



Formulation and Quality Control Tests for Nanoemulsion of Tofacitinib: A Novel Approach

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Tofacitinib (TFB) is a pioneer JAK (Janus kinase) inhibitor mainly employed to treat rheumatoid arthritis. It has proven efficacy for the treatment of rheumatoid arthritis in the oral dosage form. Oral TFB exhibited several toxic effects. Current research aims to develop a topical formulation of TFB to achieve effective treatment without any adverse effects.

Study Design: Ultrasonication Methods.

Place and Duration of Study: Sample: Swami Dayanand Postgraduate Institute of Pharmaceuticals Sciences, University of Health Sciences, Rohtak; 2020-2021.

Methods: Oleic acid, tween 80, and propylene glycol were selected as oil, surfactant, and co-surfactant, respectively. The ratio of oil:surfactant:co-surfactant was selected based on a ternary phase diagram using the aqueous titration method. The selected ratio was employed to develop eight formulations of TFB by ultra-sonication. The formulations (F1-F8) were characterized using several physicochemical methods like pH, viscosity, particle size distribution, zeta potential, drug content, and in vitro release.

Results: The formulations (F1-F8) were formulated by using the ultrasonication (high energy) method. The optimized formulation selected on the basis of characterization methods for instance, F8 possessed particle size 127.4 nm, and -18.4 (mV) zeta potential. The in vitro release of F8 was

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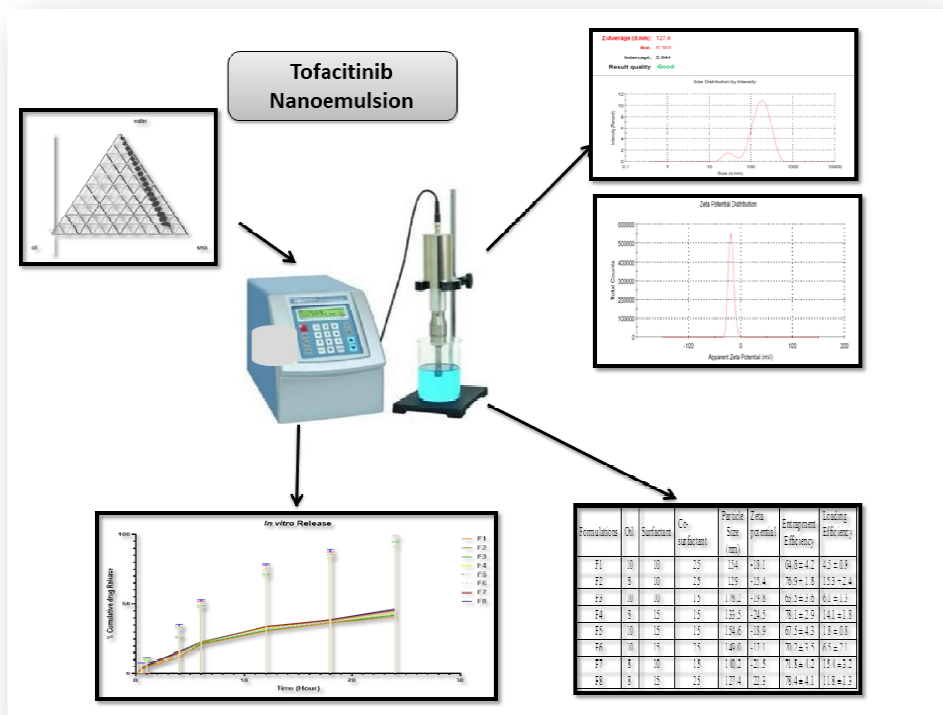
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found to be $88.1 \pm 2.5\%$ at 24 hours. It also passed the thermodynamic stability tests.
Conclusion: The current investigations conclude that tofacitinib-nanoemulsion (TFB-NE) can be used as an alternative to the oral route of TFB and is also useful in reducing the adverse effects associated with oral TFB.

Keywords: JAK; tofacitinib; phase diagram; ultra-sonication; drug content; cumulative release.

GRAPHICAL ABSTRACT



ABBREVIATIONS

- TFB : Tofacitinib;
 JAK : Janus kinase
 JAK-STAT : Janus kinase-signal transducer and activator of transcription;
 RA : Rheumatoid arthritis;
 TYK 2 : Tyrosine kinase 2;
 TFB : Tofacitinib;
 DMARDs : Disease modifying antirheumatic drugs;
 IL : Interleukins;
 IFN : Interferons;
 CRP : C-reactive protein;
 NEs : Nanoemulsions;
 TFB-NE : tofacitinib-nanoemulsion;
 PDI : Polydispersity Index;
 % EE : Entrapment Efficiency;
 % LE : Loading Efficiency;
 UV Spectrophotometer : Ultraviolet visible spectrophotometer;
 W_{total} : Weight of total drug;
 W_{free} : Weight of free drug;

W_{lipid} : Total amount of lipid;
 SD : Standard Deviation;
 PBS : Phosphate buffer.

1. INTRODUCTION

Cytokines are soluble proteins that play essential roles in a variety of biological reactions. They function as an immune system's intracellular communication mechanism, and their release and actions influence the immune response. Hematopoiesis, innate and adaptive immunity rely on the cytokine-induced JAK-STAT (Janus kinase-signal transducer and activator of transcription) pathway [1,2]. Above 50 cytokines and growth factors employ this route to send an extracellular signal to the nucleus. The dysregulation of this signaling cascade causes auto-immune disorders. JAK 1, along with JAK 3, directs cellular responses to a wide array of cytokines. Rheumatoid arthritis (RA) is one of the auto-immune disorders characterized due to disturbance in the JAK-STAT pathway. Autoimmune diseases are caused by the disruption of this signaling cascade. JAK 1 and JAK 3 regulate cellular responses to a variety of cytokines. Rheumatoid arthritis (RA) is an auto-immune disease caused by a disruption in the JAK-STAT signaling system. It is a chronic auto-immune illness found more commonly in women, causes significant disability and early death. The ultimate goal of RA treatment is to give symptomatic relief; there seems to be no known permanent cure for this disease. Treatments that target cytokine receptors have proven to be beneficial in the treatment of rheumatoid arthritis [3,4]. The activation of Janus family tyrosine kinases, often known as JAKs, has been proven to be a critical step in producing cytokines. JAK 1, 2, 3, and TYK 2 are the four molecules that make up this family. JAKs, which work in pairs, become enzymatically active when cytokines connect to their cognate receptors and phosphorylate themselves, the receptor chains, and a variety of other substrates, such as the signaling molecules and stimulation of the transcription family of inactive transcription factors [5]. TFB is a JAK1/3 inhibitor recently approved for treating rheumatoid arthritis in individuals who had failed to respond to methotrexate therapy. TFB can be used alone or in combination with methotrexate or other non-biologic DMARDs (disease-modifying antirheumatic drugs) in certain circumstances. JAK 1/3 mediated signaling of IL-2, 4, 6, 7, 15, and 21, as well as IFN- α and IFN- γ , is inhibited by TFB, resulting in inflammatory and

immunological responses being modulated. TFB can quickly lower C-reactive protein (CRP) levels in RA patients and keep them low during treatment. TFB inhibits JAK 1/3-mediated signaling of IL-2, 4, 6, 7, 15, and 21, as well as IFN- α and IFN- γ , which modulates inflammatory and immunological responses. TFB can reduce C-reactive protein (CRP) levels in RA patients immediately and maintain them during treatment. RA patients with methotrexate intolerance, TFB individual or in combination decreased bone marrow edema and slowed the course of structural damage [6-8]. Oral administration of TFB has been linked to adverse effects, severe infections like herpes zoster, immune-related disorders, upper respiratory tract infections, lymphopenia hematological disorders, urinary tract infections [9]. The topical route presents many benefits over the oral route, like smooth and incessant delivery with lowered maximum plasma concentration. This route enriches the tolerability profile as well as helps to attain comparatively sharp local drug levels. Topical TFB seems safe and well-tolerated, implying that it could be a viable alternative to oral versions [10].

Although data with robust topical efficacy of TFB is missing, topical TFB represents a promising new class of therapies for treating inflammatory skin diseases like psoriatic arthritis, alopecia areata. The current research work also focuses on the possible dermal formulation of TFB.

2. MATERIALS AND METHODS

2.1 Materials

TFB was procured from (Hector Pharmaceuticals Pvt. Ltd, Hyderabad, India), ethanol, methanol, castor oil, oleic acid, span 60, tween 80, propylene glycol, disodium hydrogen phosphate, potassium di-hydrogen orthophosphate were purchased from Loba chemicals, Mumbai. All other chemicals used were of analytical grade.

2.2 Methods

Solubility analysis of TFB was done using different oil, surfactant, and co-surfactant phases. Purified water was employed as an aqueous phase, and based on the solubility; oleic acid, tween 80, and propylene glycol were used as oil, surfactant, and co-surfactant. A pseudo-ternary phase diagram was then plotted.

2.3 Pseudo Ternary Phase Diagram Construction

To analyse the concentration range of components for nanoemulsions, the water titration method was employed to construct pseudo-ternary phase diagrams. Two-phase diagrams were constructed using the ratios 1:1, 1:2 of tween 80, and propylene glycol (S_{mix}). The oil (oleic acid) and the S_{mix} were mixed in the ratios like 1:1, 1:2, 1:3, 1:4, 1:5. All the mixtures were then diluted with distilled water, added dropwise at moderate shaking. The best oil ratio: S_{mix} was selected based on the zone of NEs in the phase diagram [10,11].

2.4 Preparation of TFB-NE

The selected ratio of oil: S_{mix} was used to make nanoemulsions using the ultrasonication process to prepare the NEs. The preparation of TFB-NE was dispersed in the oil phase and mixed into the aqueous phase by using an ultrasonicator for 20 minutes. A total of 8 formulations were made and characterized to select the best formulation [12].

2.5 Characterization of TFB-NEs

2.5.1 pH Examination

The pH of the formulations was determined at room temperature using a digital pH meter (Thermo Scientific) that had previously been calibrated with standard buffers. To avoid contamination, the electrode was thoroughly cleansed before measurement. pH measurement is necessary for topical applications to guarantee that the formulation does not irritate the skin.

2.5.2 Viscosity

At 25°C temperature, the viscosity was measured using a Viscometer IQ Air (HAAKE RheoWin Viscometer) with C35 2° spindle configuration at 0.5 to 100 rpm speed [13,14].

2.6 Particle Size and PDI Analysis

At a temperature of 25 °C and an angle of 90°, the particle size of TFB-NEs was measured in triplicate using dynamic light scattering by zeta sizer (Malvern, UK). Before analysis, all the samples were diluted 1:30 with distilled water. The software produced the graph showing the peak of particle size with PDI.

2.7 Determination of Zeta Potential

Zeta potential was measured by using a zeta sizer (Malvern UK). The purpose of this analysis was to determine how much electricity was present in the NE. The sample of NE was diluted with distilled water like the dilutions made during particle size and PDI analysis [14, 15].

2.8 Entrapment Efficiency (% EE) and Loading Efficiency (% LE)

By measuring the amount of untrapped drug in the aqueous phase, centrifugation at 5000 rpm for 25 minutes, and filtering the supernatant, the percent EE and LE of all formulations were calculated. Dilutions were also generated and examined using a UV spectrophotometer set to 286 nm. For calculating percent EE and LE, the following formulae were employed:

$$\%EE = (W_{total} - W_{free\ Drug}) / W_{total} \quad (1)$$

$$\%LE = (W_{total} - W_{free\ Drug}) / W_{total} + W_{lipid} \quad (2)$$

2.9 Drug Content Uniformity

Samples weighing 500 mg were taken and extracted using a 2:8 mixture of ethanol and water, then centrifuged for 15 minutes at 3000 rpm. Following the filtration of supernatant with 0.45 µm filter paper, the filtrate was analysed using a UV spectrophotometer at λ_{max} 286 nm. The process is performed in triplicate to avoid error and obtain precise values [16,17].

2.10 *In vitro* Release Study

The *in vitro* of TFB from TFB-NEs was analyzed using a Franz diffusion cell with a glass cylinder open at both ends. NE corresponding to 2 mg of TFB was uniformly applied to the cellophane membrane surface (which had earlier been soaked in 7.4 pH PBS for 24 hours), and the donor compartment was filled with 7.4 pH PBS. The entire assembly was placed on a magnetic stirrer maintained at 37 ± 2 °C and 100 rpm. Aliquots of 5 ml samples were withdrawn at prefixed intervals for 24 hours of study. After the collection of samples UV spectrophotometer set at λ_{max} 286 nm was used to analyze release [18].

2.11 Thermodynamic Stability Studies

Thermodynamic stability tests are critical for determining the physical stability of TFB-NE; the

following analysis was carried out to assess the thermodynamic stability of the formulations:

2.11.1 Freeze–thaw cycle (6 cycles at -25° and 25°C)

For 24 hours, the formulations were kept at a temperature of 25°C. The formulation was removed after 24 hours and stored at ambient temperature (25°C). Within 2-3 minutes, TFB-NE had restored their previous temperature. Three times, such cycles were repeated.

2.11.2 Centrifugation

After that, the NEs were centrifuged for 30 minutes at 5000 rpm and examined for creaming, cracking, and phase separation [18].

2.12 Statistical Analysis

All the values of results were expressed with mean ± SD value, n value is 3.

3. RESULTS

3.1 Preparation of TFB-NEs

From the aqueous phase titration study, the ratio of oil: S_{mix} was confirmed to be 1:1 (Fig. 1). Using 1:1 ratio, eight nanoemulsion formulations were made by employing the high-energy method, such as ultrasonication. Table 1 describes the formulae and various characterization parameters of the nanoemulsions.

3.2 pH, Viscosity, Drug Content Analysis

The pH and viscosity of all the formulations have been described in Table 2. It has been noticed that the range of pH for oleic acid-containing topical formulations is stable in the range of 5.8 ± 0.5 to 6.3 ± 0.8 . The observed pH range falls within the safe range for topical products and the pH of the skin. The viscosity ranged from 15.8 ± 3.8 to 19.5 ± 3.9 Pas, according to the results. The viscosity analysis revealed that the TFB-NEs possess finely diffused particles that represented a good flow. The drug content ranged from 63.9 ± 1.6 to 92.4 ± 3.6 as described in Table 2.

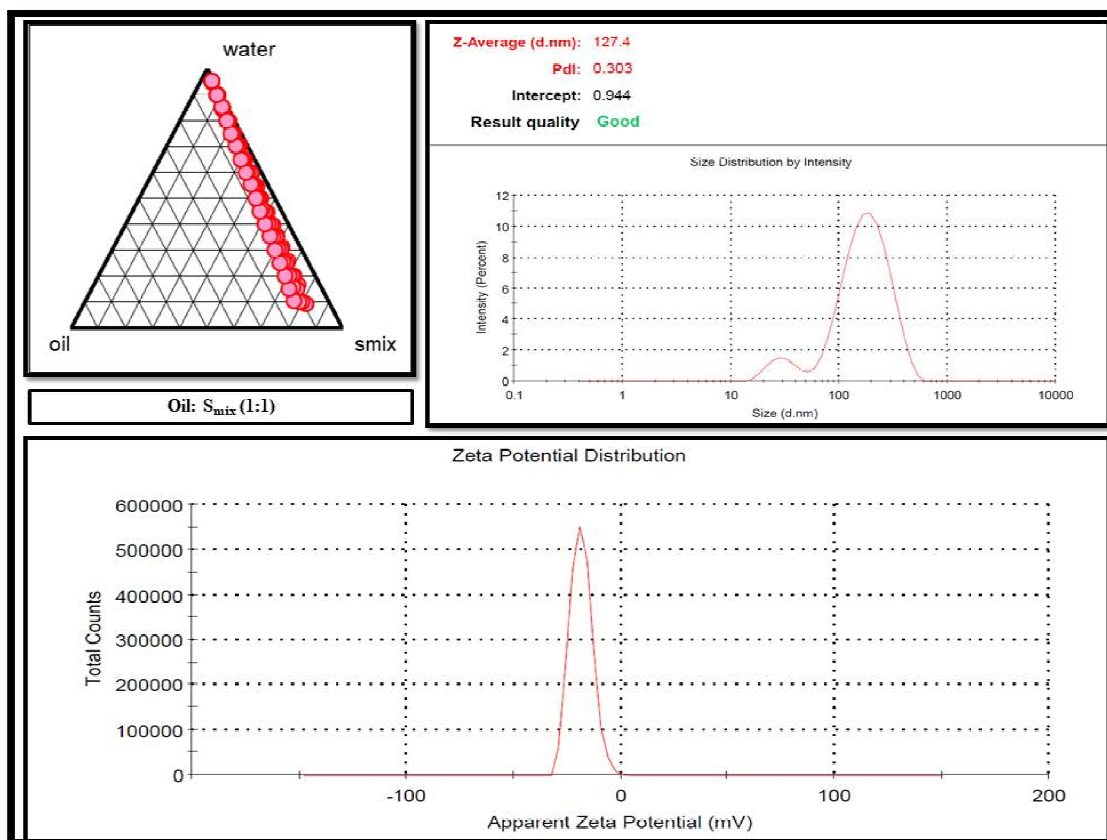


Fig. 1. Description of the phase diagram, particle size, and zeta potential

Table 1. Description of the formulation and characterization parameters of TFB-NE

Formulations	Oil	Surfactant	Co-surfactant	Particle Size (nm)	PDI	Zeta potential (mV)	Entrapment Efficiency (%)	Loading Efficiency (%)
F1	10	10	25	154 ± 3.4	0.431 ± 0.011	-18.1 ± 1.8	64.8 ± 4.2	4.5 ± 0.9
F2	8	10	25	129 ± 1.1	0.409 ± 0.022	-15.4 ± 1.2	76.9 ± 1.8	15.3 ± 2.4
F3	10	10	15	178.2 ± 1.6	0.411 ± 0.008	-19.8 ± 1.2	63.5 ± 3.6	6.1 ± 1.3
F4	8	15	15	133.5 ± 3.1	0.409 ± 0.019	-24.5 ± 3.6	78.1 ± 2.9	14.1 ± 1.8
F5	10	15	15	154.6 ± 0.9	0.327 ± 0.029	-18.9 ± 2.5	67.5 ± 4.3	1.8 ± 0.8
F6	10	15	25	149.0 ± 2.4	0.331 ± 0.024	-17.1 ± 1.8	70.2 ± 3.5	6.5 ± 2.1
F7	8	10	15	140.2 ± 2.1	0.354 ± 0.002	-21.5 ± 1.2	71.8 ± 4.2	11.8 ± 3.2
F8	8	15	25	127.4 ± 3.7	0.301 ± 0.013	-22.3 ± 3.3	78.4 ± 4.1	15.4 ± 1.3

**All the values are expressed in terms of mean ± SD, where n=3*

Table 2. Showing the results of pH, viscosity, and drug content

Formulations	pH	Viscosity (Pas)	Drug Content (%)
F1	5.9 ± 0.2	18.9 ± 4.4	74.2 ± 3.5
F2	6.2 ± 0.8	16.5 ± 5.2	88.3 ± 1.8
F3	5.8 ± 0.5	19.5 ± 3.9	63.9 ± 1.6
F4	6.2 ± 1.1	18.7 ± 3.6	78.4 ± 2.3
F5	5.9 ± 0.6	16.1 ± 2.9	65.8 ± 2.7
F6	5.9 ± 0.8	15.8 ± 3.8	69.3 ± 3.2
F7	6.3 ± 0.8	14.1 ± 5.1	89.9 ± 1.9
F8	6.1 ± 0.6	18.3 ± 3.4	92.4 ± 3.6

*All the values are expressed in terms of mean±SD, where n=3

3.3 Particle Size, PDI, and Zeta Potential

The particle size and PDI of the formulations ranged from 127.4 to 178.2 nm and 0.303 to 0.431, respectively. The value of PDI is less than 0.7, which indicates the uniform and tapered distribution of particles. The size range also suggested the adequate particle size of formulation for topical delivery of nanoemulsion. Further, zeta potential varies from -15.4 to -24.5 mV, indicating the stability of surface charge potential and the lesser tendency of the formulation to form aggregate (Fig. 1). Table 1 describes the value of particle size and zeta potential.

3.4 In-vitro Release Studies

The % cumulative release of formulations F1 to F8 was described in Fig. 2, which showed

the *in vitro* release behavior of all the formulations in phosphate buffer (PBS) pH 7.4. The F8 formulation exhibited maximum *in vitro* release at 24 hrs, i.e. 88.1 ± 4.8 in contrast to other formulations. This indicated the significance of tween 80 and propylene glycol as surfactant and cosurfactant, respectively, in enhancing drug release. Tween 80 possesses the capability to alter the physicochemical assets of skin upon interaction with phospholipid bilayer, allowing the drug to penetrate deeply [19].

3.5 Thermodynamic Stability Testing

Physical stability testing revealed no substantial changes in the formulation, and centrifugation revealed no symptoms of phase separation. The results of thermodynamic stability studies are described in Table 3.

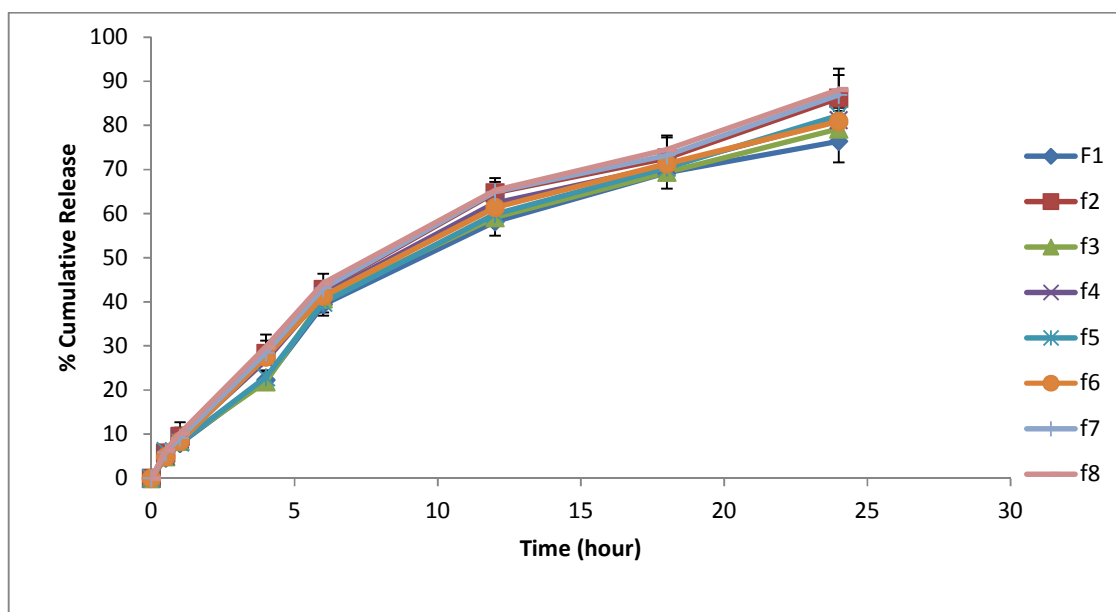


Fig. 2. % Cumulative release of TFB from formulations (F1- F8)

Table 3. Showing results of thermodynamic stability studies

Physical Stability Tests	Particle Size	pH	Drug Content	Phase Separation
Freeze–thaw cycle	127.6 ± 2.2nm	6.2± 0.4	91.67 ± 2.9 %	Not observed
Centrifugation	No significant change observed			

4. DISCUSSION

In November of 2012, TFB became a pioneer as a JAK block for the treatment of RA. TFB has been shown to suppress cytokine signaling in a variety of ways. It is a powerful inhibitor of JAK 1 and 3, inhibits interleukins (IL-2, 4, 7, 9, 15, and 21) and interferons (IFN- α/β , IFN- γ). JAK 2 and Tyk 2 (tyrosine kinase) are inhibited by TFB at a negligible level [20,21]. Further, it impairs STAT (Signal Transducer and Activator of Transcription), which impacts the expression of genes that regulate cellular functions such as immunological responses and hematopoiesis. In comparison to methotrexate, TFB provides superior joint and infected synovial tissue salvage, and it is also compatible with methotrexate, resulting in a better combination. On the other hand, the adverse effects of TFB are inescapable. Little data is available on the topical approaches of TFB, which could aid in eradicating or at least reducing the frequency of side effects. With the topical method, less medication enters the systemic circulation and is more localized in the skin, providing localized action [22-24]. By developing a topical formulation of TFB, the complications of oral TFB can be avoided since the immunogenic reactions will be limited. TFB meets nearly all of the criteria for developing a topical formulation, including low molecular weight, moderate partition coefficient, and half-life [25-27]. The use of oleic acid, tween 80, and propylene glycol for the current formulation is significant. By disrupting the lipid framework of the stratum corneum and permitting drug permeability, Oleic acid can operate as a percutaneous absorption enhancer for a variety of medicines. Further, it is obtained from natural sources and is biodegradable; hence it is compatible with the human skin [28,29]. Tween 80 is a non-ionic hydrophilic surfactant employed for the emulsification and dispersion of components. It also aids in reducing the particle size and increasing the drug loading efficiency of the nanoemulsions [30,31]. Propylene glycol helps increase entrapment efficiency and *in vitro* permeation of the drug molecules [32,33].

The NE formulations F1-F8 possessed pH in the range of 5.8 ± 0.5 to 6.3 ± 0.8 , confirming their suitability for the skin as highly acidic or alkaline pH may cause skin irritation or erythema. The particle size of all the formulations varied from 127.4 ± 3.7 to 178.2 ± 1.6 nm. Small size facilitates the permeability across the stratum corneum. The size of the nanoemulsion particles had a significant impact on the transdermal transit. Nanoemulsions with small particle size can diffuse easily into the epidermis however, larger particle size nanoemulsions cannot penetrate the stratum corneum and travel only along the hair follicle canals. The stability of the NE is shown by a zeta potential range of + 25 mV to -25 mV; the zeta potential of all formulations was found in this range [34,35]. The % EE, % LE, and drug content of F8 were $78.4 \pm 4.1\%$, $15.4 \pm 1.3\%$, and $92.4 \pm 3.6\%$, respectively, which revealed the highest amount in contrast to other formulations. Further, the *in vitro* release of F8 was found to be $88.1 \pm 4.8\%$, which is the maximum compared to other formulations. The maximal release from F8 suggested that the composition of F8 is highly effective in the permeation of TFB from the skin. The thermodynamic stability studies had also exhibited the stability of F8 during the freeze-thaw cycle and centrifugation; no significant change was observed in the thermodynamic stability studies afterwards. The quality control tests like particle size, PDI, zeta potential, drug content (%), % LE, % EE, and *in vitro* release of the TFB-NE have been performed and resulted in the optimized formulation [16]. Hence, based on the outcomes of the physicochemical characteristics of TFB-NE, it may be specified that nanoemulsion can serve as a better dosage form alternative for the topical delivery of TFB. It also aids the skin permeation without imparting ill effects of TFB as observed in oral delivery of TFB. As little data is available for the topical delivery of TFB, there is more need to research the off label, *i.e.* topical delivery of TFB. Although the current TFB-NE formulation exhibited good *in vitro* release behavior, there is necessary to evaluate the permeation of TFB-NE through *in vivo* studies following standard animal study protocols.

5. CONCLUSION

The present study indicated that a nanoemulsion formulation containing TFB was successfully formulated using the high energy method *i.e.* ultra-sonication. The topical delivery of TFB is not observed frequently for any diseases, however, for psoriatic arthritis, alopecia areata it has been employed in topical form like ointment, and solution. The current study used oleic acid, tween 80, and propylene glycol as oil, surfactant, and co-surfactant, respectively, to establish a novel strategy for topical delivery of TFB. The topical TFB-NE may provide therapeutic benefits without any adverse impacts on the body, unlike oral TFB. The results of various characterization approaches proved the significance of topical nanoemulsion of TFB in topical delivery. Further, studies on animals for anti-arthritic activity need to be performed for TFB-NE.

DISCLAIMER

The goods employed in this study are widely used in our field of study. There is no conflict of interest between the authors and producers because we do not plan to use them as a tool for litigation, but rather to further knowledge. Furthermore, the research was not supported by the production firm, but rather by the author's own personal efforts.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENT

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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